THE WEAKENING ACTION OF THIOCTIC ACID IN UNYEASTED AND YEASTED DOUGHS[1]

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ABSTRACT

Reduced thioctic acid added to flour in various quantities, followed by mixing on the farinograph, resulted in weakened mixing behavior — an effect not obtained with cyclic thioctic acid. Addition of cyclic thioctic acid to yeasted systems, however, gave the effect of reduced thioctic acid, thus demonstrating the potency of the thioctic-reducing enzyme system known to be present in yeast. Baking data revealed flour to be sensitive to exogenous thioctic acid levels as low as 0.025 μmole per g. of flour. In unyeasted systems, dithiothreitol produced effects similar to those of reduced thioctic acid. Cyclic dithiothreitol, however, was inactive in yeasted systems, thus demonstrating the enzyme specificity of the yeast enzymes. Incubation of flour slurries containing reduced thioctic acid resulted in increased amounts of water-soluble and acid-soluble proteins.

In previous publications from this laboratory (1,2) and elsewhere (3) the presence of thioctic acid in flour is reported. Initial consideration had been given to the possible improver action of thioctic acid monoxide. The evidence reported here indicates that significant dough-

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weakening is caused by small quantities of reduced thioctic acid ($CH_2SHCH_2CHSHCH_2CH_2CH_2CH_2COOH$). The presence of a thioctic-reducing enzyme system in yeast (4) probably ensures constant replenishment of reduced thiotic acid in a fermenting system, thus extending the effect of minute quantities, which would be catalytic.

In a recent publication (5), the powerful reducing effect of dithiothreitol ($CH_2SHCHOHCHOHCH_2SH$) on protein disulfide linkages as compared with other thiols, including reduced thioctic acid, is reported. Reduced thioctic acid possesses structural similarity to dithiothreitol and has reducing power of a similar order of magnitude.

The favored theory on the mechanism of dough-weakening caused by thiols is that –SH interchange with protein disulfide groups results in cleavage of large gluten molecules, with accompanying structural weakening. This theory still is subject to testing and proof. Regardless of the mechanism, it is plausible that reduced thioctic acid should be an effective dough-weakener, equal to, or greater than, the thiols.

The data reported here demonstrate the sensitivity of unyeasted doughs to dithiothreitol and reduced thioctic acid. Fermenting systems were found to be sensitive to cyclic thioctic acid, but insensitive to cyclic dithiothreitol, thus demonstrating the enzyme specificity to thioctic acid. The low levels of thioctic acid which have a noticeable effect on fermenting systems suggest that the level of endogenous thioctic acid may be one important criterion of flour strength. It is noteworthy that the flour used in these experiments had a very low level of thioctic acid.

Materials and Methods

Flour. An untreated, hard red spring flour, analyzing 0.45% ash and 14.6% protein, was employed.

Dithiothreitol was obtained from California Biochemical Corporation.

Thioctic acid was obtained from the Mann Research Laboratories, Inc.

Reduction of Thioctic Acid. Thioctic acid was reduced under mild conditions by dissolving it in 80% acetic acid and shaking in the presence of zinc. Progress of the reduction could be followed by removing portions of the solution at intervals and measuring the absorbance at 334 mμ, an absorbance peak of cyclic thioctic acid. The reduced thioctic acid was extracted from the mixture with diethyl ether. The ether extract was washed with 0.1N hydrochloric acid and assayed for reduced thioctic acid by titration with an ethanolic solution of iodine. To impregnate the reduced thioctic acid on a carrier, the ether extract was slurried with wheat starch and this was subjected to vacuum to remove the solvent. The preparation was stored in a refrigerator. Similarly, cyclic thioctic acid was impregnated on a starch carrier and stored.

Oxidation of Dithiothreitol. Cyclic oxidized dithiothreitol was prepared according to the procedure given by Cleland (5). The pure crystalline material was used as such.

Unyeasted Dough Farinograms. Successive farinograms were run with the addition of reduced and cyclic thioctic acid and dithiothreitol.

Appropriate quantities of reduced and cyclic thioctic acid on their starch carriers were mixed with the dry flour in the bowl prior to the addition of absorption water. The dithiothreitol was dissolved in a portion of the absorption water and added to the flour in the bowl, followed by the remainder of the water.

Yeasted Dough Farinograms. A suspension of 2 g. of yeast, 0.5 g. of sugar, and 8 ml. of water was incubated for 50 min. at 30°C. and added to the flour in the farinograph bowl prior to the addition of the remaining absorption water.

Successive farinograms were run with cyclic thioctic acid and cyclic dithiothreitol which had been added to the yeast suspensions at the time of incubation.

Effect of Reduced Thioctic Acid on Extractable Protein. Flour-water slurries (1:3) containing various levels of reduced thioctic acid were incubated at 30°C. for 1 hr. One set of slurries was centrifuged and the water-soluble extracts were analyzed for protein by the Kjeldahl method. Another set of slurries was made 0.05*N* with acetic acid and centrifuged; the acetic acid-soluble extracts were analyzed for nitrogen.

Estimation of Endogenous Thioctic Acid in Flour. A water extract of flour was prepared by allowing a flour-water slurry (1:4) to stand for 1 hr. at room temperature and then centrifuging it. This was made 1.0*N* with hydrochloric acid, hydrolyzed for 3 hr. on a steam bath, and extracted with chloroform. The chloroform extract was extracted twice with 5% sodium bicarbonate. The bicarbonate solution was acidified and extracted with diethyl ether. The ether extract was dried over sodium sulfate and reduced to dryness *in vacuo*. The residue was taken up in chloroform, applied to a wide strip of Whatman No. 1 filter paper, and chromatographed in 1% acetic acid. A wide band centering on the R_f value of thioctic acid (0.7 in 1% acetic acid) was cut from the chromatogram, extracted with chloroform, reduced under vacuum, and then spotted on another chromatogram. Thioctic acid was detected by immersion in 0.1*N* potassium permanganate. Comparison of this spot with known quantities of synthetic thioctic acid on other chromatograms indicated that the level of endogenous thioctic acid in this flour was about 0.5 p.p.m.

Baking Data

In all doughs, the cyclic thioctic acid impregnated on starch was mixed with the flour before any of the other ingredients. The cyclic dithiothreitol was dissolved in a small portion of the absorption water and mixed with the flour before any of the other ingredients. Straight doughs were made, with the following formula: 100% flour, 64% water, 2% yeast, 2% salt, 8% sugar, and 5% nonfat dry milk. Optimum mixing was 12 min. on a Hobart A-120 mixer. Fermentation was 1 hr. and 30 min. to the first punch and then 30 min. The dough was rounded and then rested 20 min. before being moulded. The loaves were proofed at 100°F. to 1 in. above the pan and then baked for 28 min. at 450°F.

Results and Discussion

As seen in Fig. 1, the thiols, reduced thioctic acid and dithiothreitol,

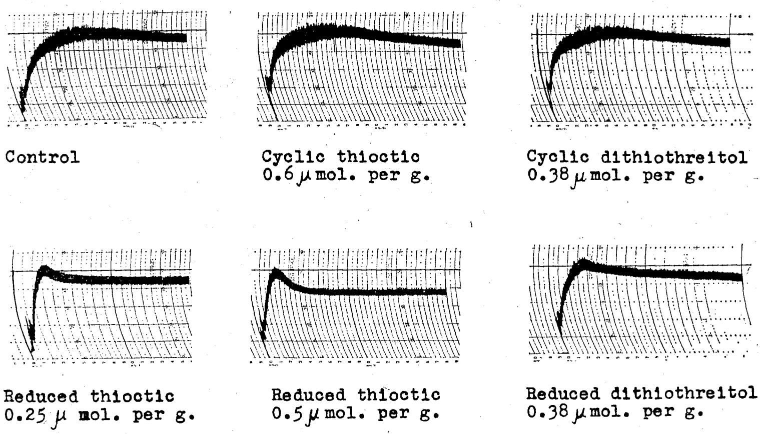

Fig. 1. Unyeasted farinograms. Effect of reduced and cyclic (oxidized) forms of thioctic acid and dithiothreitol on unyeasted flour-water doughs.

weakened the mixing characteristics of the flour. This is a typical effect of thiols (6,7,8). The flour was sensitive to reduced thioctic acid at a level less than 0.15 μmole per g. A level of 0.5 μmole per g. was completely destructive. At these levels, cyclic thioctic acid had no effect.

As seen in Fig. 2, the incubation of thioctic acid in a yeast-sugar suspension prior to its addition to flour at the farinograph produced an effect like that of reduced thioctic acid. The same experiment using

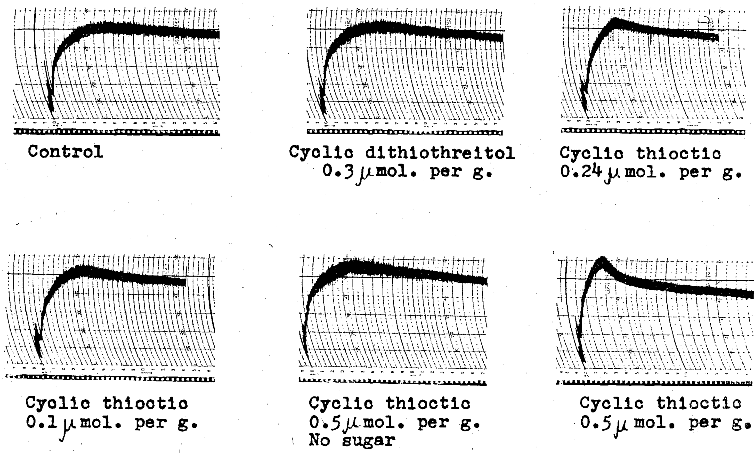

Fig. 2. Yeasted farinograms. Effect of cyclic thioctic acid and cyclic dithiothreitol on yeasted doughs.

cyclic dithiothreitol did not give the same effect as reduced dithiothreitol. Thus, it was demonstrated that the thioctic-reducing enzyme system in yeast reported by Black *et al.* (4) effectively reduced thioctic acid, but not cyclic dithiothreitol. The enzyme is specific for the naturally occurring substrate, thioctic acid. Also, an actively fermenting yeast system is required, as evidenced by the lack of effect when sugar was absent.

The baking data, Table I and Fig 3, confirm the results seen on the farinograms and demonstrate a very high sensitivity of bread dough to thioctic acid. Increments of 0.025 μmole per g. (5 p.p.m.) produced discernible differences in baking results. If the dough-weakening mechanism of reduced thioctic acid involves its oxidation or cyclization, the

TABLE I

EFFECT OF CYCLIC THIOCTIC ACID AND CYCLIC DITHIOTHREITOL ON BAKING PROPERTIES

FLOUR TREATMENT	GRAIN	VOLUME	
μmol./g. flour		*cc.*	%
Untreated control	100	2,500	100
Cyclic thioctic acid			
0.025	96	2,350	94
0.050	95	2,275	91
0.150	90	1,925	77
0.300	80	1,425	57
Cyclic dithiothreitol			
0.150	101	2,450	98

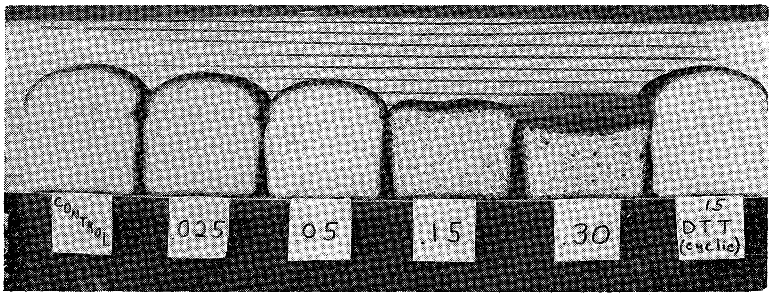

Fig. 3. Effect of cyclic thioctic acid and cyclic dithiothreitol (labeled DTT) on baking performance; the concentrations given are μmoles per g. of flour.

reducing-enzyme system of the yeast would be able to reduce it as it was cyclized. Thus, it is conceivable that thioctic acid functions catalytically, indirectly subjecting flour proteins or components to the reducing action of yeast.

As seen in Fig. 4, increased amounts of water-soluble protein were

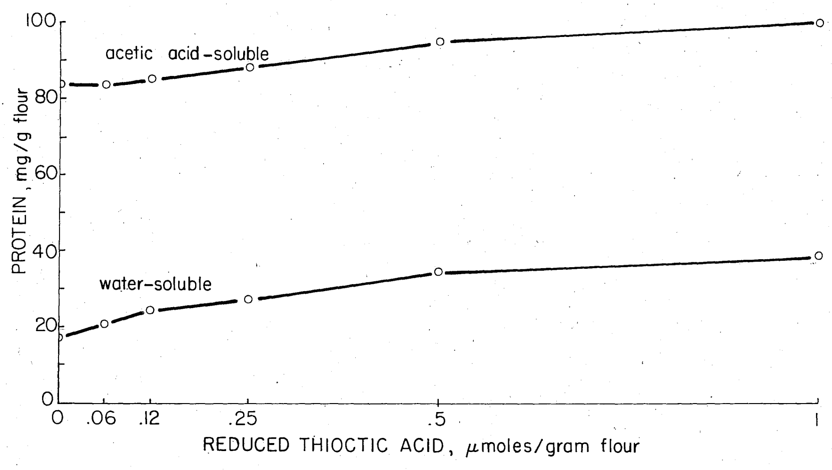

Fig. 4. Effect of reduced thioctic acid on solubility of flour proteins in water and 0.05M acetic acid, after incubation as a slurry (1:3) at 30°C. for 1 hr.

obtained after the flour was incubated with increments of reduced thioctic acid as low as 0.06 μmole per g. An accompanying increase also was observed in the amount of protein extractable by 0.05M acetic acid. A close comparison of data reveals that the rate of increase of water-soluble protein exceeded the rate of increase of protein extractable by 0.06M acetic acid. It is likely, then, that the reduced thioctic acid forms at least some water-soluble protein from protein that originally was water-insoluble but acetic acid-extractable.

A comparable result was noted by Mecham *et al.* (9) when observing the effect of the blocking agent, N-ethylmaleimide (NEMI), on the mixing behavior of doughs. An increase in the amount of protein extractable by $0.01N$ acetic acid was found to occur with the dough-weakening effect of NEMI. Meredith and Bushuk (10) found that a decrease in the amount of gel protein of dough occurred with the dough-weakening effect of NEMI. Also, Meredith and Hlynka (8) found this to occur with the dough-weakening effect of thiols.

The mechanism by which reduced thioctic acid and other thiols weaken doughs is still subject to study. Currently, work is being conducted in this laboratory which should indicate if gluten-protein fragmentation by sulfhydryl-disulfide interchange is primarily responsible for this increased solubilization or if some other type of change occurs. Sullivan and Dahle (11) recently reported data which suggest that sulfhydryl-disulfide interchange may not be involved in the improver mechanism of formamidine disulfide.

The very low levels of endogenous thioctic acid in the strong flour used may suggest a connection between endogenous thioctic acid levels and flour strength characteristics. This will be investigated.

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